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AWARD NUMBER: W81XWH-05-1-0524

TITLE: Prostate Derived Ets Factor, an Immunogenic Breast Cancer Antigen

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REPORT DATE: September 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-09-2006	Annual	1 Sep 2005 – 31 Aug 2006

4. TITLE AND SUBTITLE

Prostate Derived Ets Factor, an Immunogenic Breast Cancer Antigen

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0524

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Health Research, Incorporated
Buffalo, New York 14263

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of the proposed research was to test the following concept. Due to prostate restricted expression of Pse in normal tissues of mice, Pse is likely to be immunogenic in female mice. T cell responses of male and female mice to Pse and a control antigen Her-2/neu were compared. Two different assays were used for this purpose; the ELISPOT assay and the Cytotoxicity assay. To date, we have found that in the FVB strain of mice immunization with Pse induces specific T cell responses in female mice but not in male mice. In contrast, Her-2/neu is immunogenic in both female and male mice.

These results are novel and significant and they support our concept and suggest that PDEF (the human homologue of Pse) is likely to be similarly immunogenic in female breast cancer patients. Our results also suggest that lack of immunogenicity of Pse in male mice is likely due to some mechanism of tolerance to a self protein since Pse/PDEF is strongly expressed in the normal prostate tissue of males.

15. SUBJECT TERMS
Prostate specific Ets, Female specific immunogenicity, A candidate breast cancer antigen

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	6	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION: Prostate derived Ets factor is a relatively novel member of the Ets family of transcription factors (1). It is also a unique since it shows most restricted expression in normal human tissues that is primarily limited to normal prostate with weaker expression in normal bronchus/trachea tissue (2-4). Similarly, Pse (prostate specific Ets, the mouse homologue of PDEF also shows highly restricted expression in normal mouse tissues that is primarily restricted to normal prostate and colon tissues (5). Based on this understanding, we proposed that Pse is likely to be more immunogenic in female mice than in male mice, and the test of this concept was the focus of our research.

BODY: Our first task was to immunize male and female mice with Pse expression plasmid and enumerate total T cell responses and cytotoxic T cell responses using the ELISPOT assay and ^{51}chromium release assay. This experiment was performed and results of representative ELISPOT images are shown in Figure 1. Our data show that in contrast to female mice, male mice fail to induce Pse specific IFN-γ secreting T cell responses. The average number (from triplicate wells) of IFN-γ secreting T cells from Pse immunized female (panel A) and male mice (panel C) was 143 and 13 respectively. The latter number is similar to that observed with negative control, i.e., vector immunized mice (panels B and D).

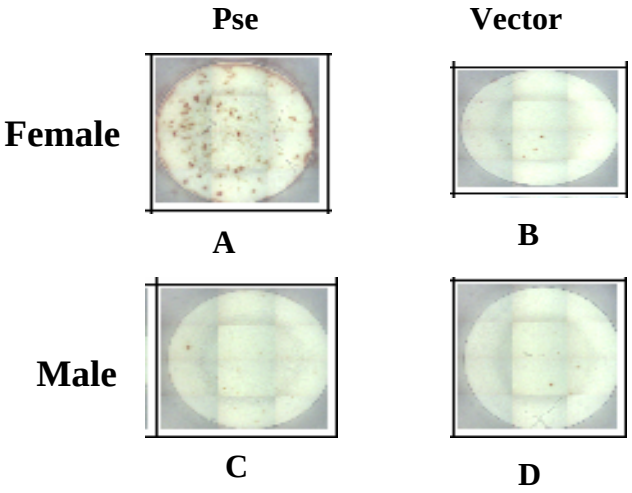

Figure 1. Testing of Pse specific cellular immunity by ELISPOT assay for cytokine IFN-γ. Upper panels A and B respectively show representative images of individual wells showing IFN-γ secreting T cells from Pse-transfected dendritic cell (Pse- DC)- immunized (A) and vector-DC-immunized (B) female mice. The lower panels C and D respectively show data for similarly immunized male mice.

A comparison of the Pse specific cytotoxic T cell response by ^{51} Cr release assay is shown in Figure 2

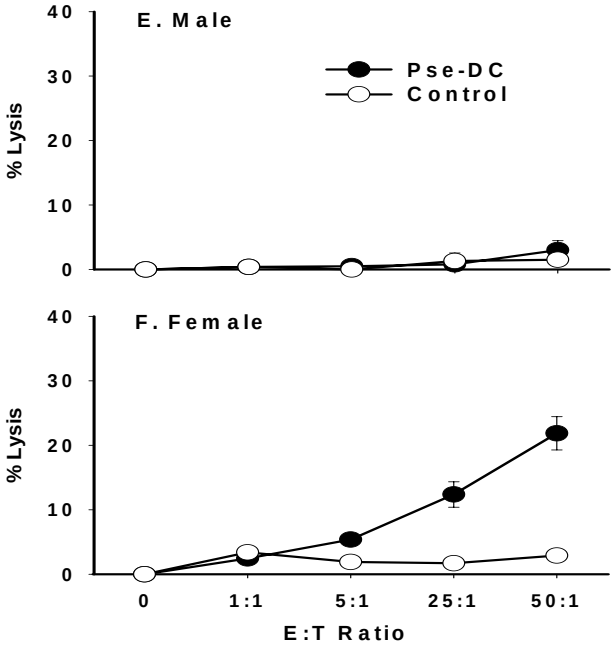

Figure 2. Comparison of Pse specific CTL responses in male and female mice. Specific lysis of Pse-positive targets from a syngeneic mammary tumor cell line was observed with splenocytes from Pse-DC immunized female mice (solid circles in lower panel) but not from male mice (solid circles in upper panel). As control, both male and female mice immunized with vector plasmid failed to elicit Pse-specific CTL responses (open circles in top and bottom panels).

The above experiment was repeated once in the same FVB strain of mice and similar results were obtained (data not shown).

Together, the data shown in figures 1 and 2 support our concept that due to prostate restricted expression of Pse, it is immunogenic in female FVB mice but not in male FVB mice.

In task 2, we proposed to clone the neu oncogene and compare the response of male and female mice to this antigen. To that end, we obtained the Her-2/neu oncogene expression plasmid from colleagues in the field and tested male and female mice for their responses to this antigen. Representative images of the ELISPOT assay from male and female FVB mice are shown in Figure 3. The average number of IFN-γ secreting T cells/ 10^6 splenocytes from triplicate wells from Her-2/neu immunized female and male mice was 152 and 98 respectively. These data show that both female and male mice respond to immunization with Her-2/neu antigen, although female mice show a relatively more robust response than male mice.

Female **Male**

In task 3, we proposed to compare Pse and neu specific T cell responses. These results are shown below in figure 4.

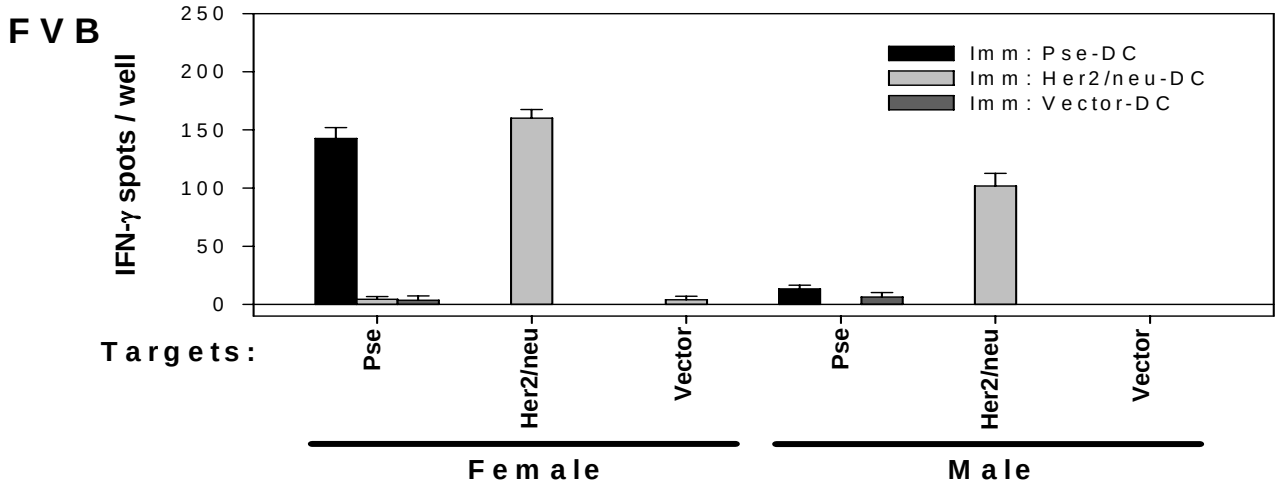

Figure 4. A comparison of Pse and Her-2/neu specific T cell responses induced in female and male mice of FVB strain Spleens were harvested from female/male FVB mice immunized three times with Pse-DC, Her2/neu-DC or Vector-DC respectively. After 5 day culture in the present of stimulator and IL-2, the percent of Pse-specific IFN-g secreting T cells were determined by ELISPOT using Pse-DC, Her2/neu-DC and Vector-DC as target cells.

From experiment shown in Figure 4, we find that whereas Pse specific immune response was seen only in female mice, both male and female mice induced specific T cell responses to control Her-2/neu antigen. These results strongly support our hypothesis that lack of Pse specific immune response in male mice is likely due to some mechanism of tolerance to Pse as a self protein in male mice.

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We are now testing the C57BL/6 strain of mice to determine whether or not Pse specific T cell responses can be similarly induced in female mice but not in male mice.

Key Research Accomplishments:

- Demonstrated that Pse is immunogenic in female FVB mice but not in male mice
- In contrast, Her-2/neu is immunogenic in both female and male mice.

Reportable outcomes: We expect to present this work at upcoming national and international meetings on cancer. This work was also part of a new grant application submitted to Genentech Inc. in which one of the aims is to determine the immunogenicity of PDEF in the context of human HLA-A2 antigen. Also, this work will be the basis of an idea development grant application to DOD and another grant application to National Cancer Institute in the near future.

Conclusions: Above results are novel and significant and they support our concept and suggest that PDEF (the human homologue of Pse) is likely to be similarly immunogenic in female breast cancer patients. Our results also suggest that lack of immunogenicity of Pse in male mice is likely due to some mechanism of tolerance to a self protein since Pse/PDEF is strongly expressed in the normal prostate tissue of males. Understanding the mechanism of tolerance to Pse and developing approaches to overcome it will be useful for similar efforts at breaking tolerance against PDEF in male breast cancer patients.

References:

1 Oettgen, P., Finger, E., Sun, Z., Akbarali, Y., Thamrongsak, U., Boltax, J., Grall, F., Dube, A., Weiss, A., Brown, L., Quinn, G., Kas, K., Endress, G., Kunsch, C. and Libermann, T. A. PDEF, a novel prostate epithelium-specific ets transcription factor, interacts with the androgen receptor and activates prostate-specific antigen gene expression. J. Biol. Chem.; 275: 1216-25, 2000.

2 Ghadersohi, A., Sood, A. K. Prostate epithelium-derived Ets transcription factor mRNA is over expressed in human breast tumors and is a candidate breast tumor marker and a breast tumor antigen. Clin. Cancer Res. *7*: 2731-38, 2001.

3. Geradts, J., Saxena, R, Groth, J, Desouki, M., Cheewakriangkrai, C., Rodabaugh, K., and Sood, AK. Frequent overexpression of prostate derived ets factor (PDEF) protein in breast cancer progression. AACR Meeting Abstracts; 2006: 780. Abstract #3319.

4. Sood AK, Saxena R, Groth R, Desouki M, Cheewakriangkrai C, Rodabaugh KJ, Kasyapa, CS and Geradts J. PDEF protein is frequently over expressed in breast and prostate tumors and is a candidate novel marker and target in these cancers. Submitted for publication.

5. Yamada N, Tamai Y, Miyamoto H, Nozaki M. Cloning and expression of the mouse Pse gene encoding a novel Ets family member. Gene. 241:267-274, 2000.

Appendices: None

Supporting data: None